CXXII. SULPHUR DISTRIBUTION IN THE COMPONENT STRUCTURES OF WOOL AND PORCUPINE QUILLS.

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The views originally advanced by one of us [King, 1926] and in more detail by Barritt and King [1929] regarding the biological significance of sulphur variability in wool are, in brief, that, in the first place, "keratinisation" is the result of incorporation of cystine nuclei with the primary plasmic cell-forming material, the extent of this incorporation or "sulphur stimulus" being variable with different types of wool and with different genetic, pathological, and environmental factors, particularly, as regards the last named, diet and seasonal conditions. Further evidence of this variability has been published by Rimington [1929] and Barritt and King [1929] as opposed to the statement of Robertson and Marston [1929] that wool keratin has a constant sulphur content.

Recent work in these laboratories by Bonsma [1931] has established some evidence of correlation of sulphur content with diet and seasonal changes in the case of South African wool, and his discussion of its biological and technical significance is substantially an elaboration of the views already advanced [King, 1929].

Secondly, from indirect evidence, summarised in the first paper above mentioned (details of which are published for the first time in the accompanying note [Barritt and King, 1931]) it was concluded that "the medulla cells...are substantially devoid of sulphur" and that a biological deficiency in "sulphur stimulus" is a factor associated with presence of the medulla, unkeratinised by cystine incorporation, which constitutes the inner core of kempy fibres.

The essential evidence lies in the substantially lower sulphur content of medullated fibres as compared with non-medullated fibres from the same portion of the fleece, and, in view of the fact that recently Rimington [1931] found only comparatively small differences in this respect with certain types of wool, it appeared desirable to examine some hirsute tissue in which the medulla could be separated with some degree of accuracy and independently analysed.

For this purpose porcupine quills, morphologically analogous to wool fibre,

were employed. They were split with a knife, and the medullated core carefully scraped away from the cortex. Complete separation is impracticable on account of the radially disposed lamellae of cortical tissue (see Fig. 1*a*) but, while the medulla remained contaminated with the cortical lamellae, the cortex was obtained substantially free from medulla.

The samples were degreased with boiling benzene, washed in water, and dried to constant weight.

Analysis gave the following results[1].

Ash (whole quill) 0·79 %.

	Whole quill	Cortex	Medulla
Total sulphur	1·31	1·46	1·25
	1·38	1·53	1·23
Mean	1·35	1·50	1·24

The sulphur content for the whole quill is thus 10 % lower than that for the cortex. Typical values for non-kempy and kempy wool fibre (3·82 and 3·33 respectively) show a difference of 12·8 %, a figure of the same order. The actual weight of medulla substance in the wool kemp examined is approximately only 5 % of the total fibre weight. Unfortunately, as mentioned above, the failure to obtain complete separation of the porcupine medulla again precludes direct analytical evidence of its freedom from sulphur, but the microscopic appearance of its very attenuated structure of hollow cells indicates that its proportion by weight of the whole quill must be very small, and the results therefore are not inconsistent with those for medullated wool fibre.

Confirmatory evidence was sought from the action of specific staining agents on sections of both wool and quill.

Sodium plumbate solution (10 minutes treatment in the cold) rather disturbs the structure on account of its alkaline character, but shows clearly the location of the sulphur in the cortex (Fig. 1*b*).

The Sullivan reagent (applied by boiling the quill section in dilute hydrochloric acid for a few minutes to liberate free cystine, and then immersing in sodium naphthaquinone-4-sulphonate) shows a slight diffusion of stain, which is however again essentially confined to the cortex (Fig. 1*c*).

The Pauly reagent (diazotised sodium sulphanilate) not only distinguishes clearly the structural form of the medulla from the lamellae of the cortex, but indicates chemical differences as well.

This reagent is well known to give similar brown-red colorations with a number of the naturally-occurring amino-acids, but according to Totani [1915] when the stain originally developed is treated with zinc and hydrochloric acid, with subsequent addition of ammonia until alkaline, only the tyrosine and histidine stains remain, the former rose-red, the latter golden yellow. Moreover on oxidation of these secondary colorations with hydrogen peroxide, the tyrosine colour disappears leaving only a lemon-yellow histidine coloration,

[1] We are indebted to Dr C. Rimington for these analyses.

which is stable to this oxidising agent. Convenient practical details for these tests are given by Burgess and Rimington [1929].

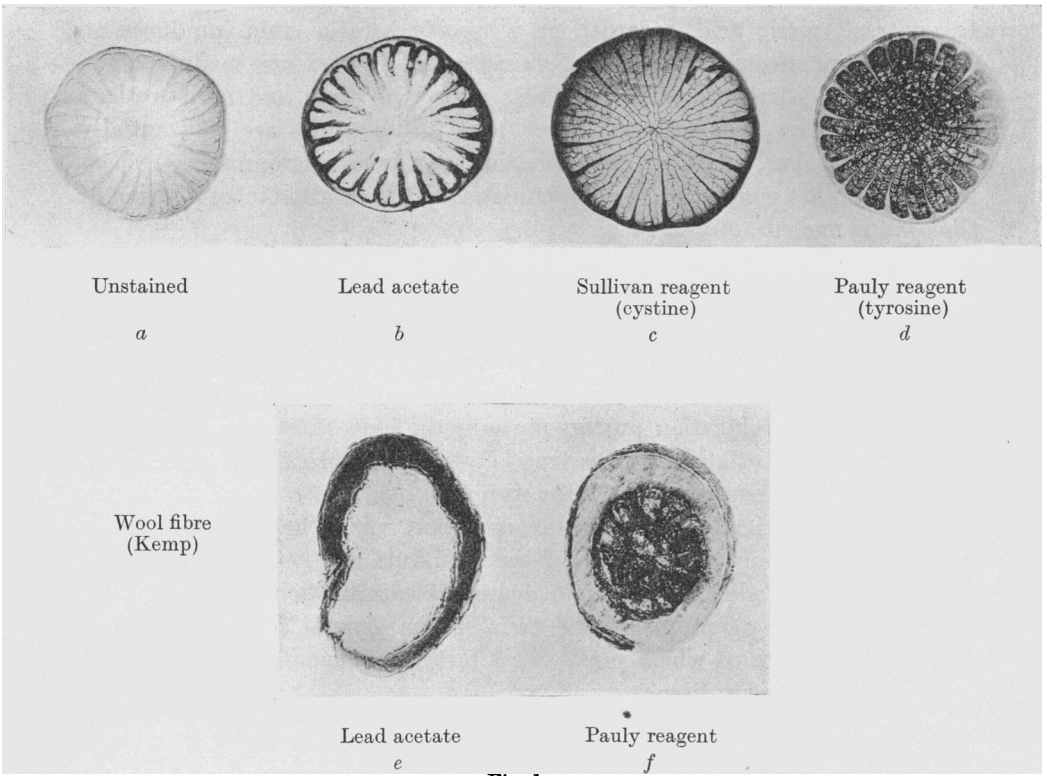

Fig. 1.

With sections of non-medullated wool fibres these colour changes are readily followed, the final lemon-yellow histidine stain being distributed throughout the cortex. Sections of medullated wool fibre however behave anomalously, in that the pronounced red-brown stain of the medulla is resistant both to the reducing and oxidising actions. Thus elimination of the tyrosine stain leaves a more brownish stain, equally persistent with the histidine stain remaining in the cortex, and masking any residual histidine stain which may also be given by the medulla (Fig. 1*f*).

Porcupine quills show similar behaviour, though the residual histidine stain of the cortex is very faint compared with that of wool fibre (Fig. 1*d*).

The photographs of course give a very inadequate impression of the striking colour differences.

Thus the chemical distinctions in the various components of wool fibre may be summarised:

Scales. Cystine, positive; tyrosine (and ? histidine), negative.
Cortex. Cystine, positive; tyrosine and histidine, positive.

Medulla. Cystine, negative; tyrosine (and ? histidine), positive; also specific reaction to Pauly reagent not shown by scale or cortex, indicating some constituent, as yet unidentified, peculiar to the medulla.

In contrast, picric acid, regarded as a specific keratin stain, produces no distinguishing effects, but a general stain for scale, cortex and medulla alike.

Thus the differential staining effects with both wool and quill sections confirm the view previously advanced that medulla cells are substantially devoid of sulphur, and that keratinisation, so far as scleroproteins such as wool keratin are concerned, is characteristically the result of incorporation of the cystine nucleus.

We therefore prefer to regard the term "keratinisation" as synonymous with cystinisation in contrast to general hardening or cornifying effects occurring in protein structures.

If we accept the histological view that scale, cortex, and medulla are all developed from the same primary plasmic material of the basal layer of the epidermis, the cornification process must be different in each case. That of the scale cells essentially involves true keratinisation or cystinisation, apparently without incorporation of the tyrosine nucleus; that of the cortex involves both cystine and tyrosine incorporation, while the hardening of the medulla cells, mainly a drying up of the semi-fluid contents to form hollow cells, involves in addition a certain degree of cornification associated with some keratinising agent (using the term in the general sense) other than cystine, the nature of which must await further chemico-histological study of the follicle and skin tissues.

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